Expression of mucins in normal salivary glands and mucoepidermoid carcinoma of salivary glands.

Matilda Llupi

Rabije Qoku

Supervisor:
Gunnar Warfvinge, Dept. of Oral Pathology.

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Malmö University
Faculty of Odontology
205 06 Malmö
ABSTRACT

Mucoepidermoid carcinomas (MECs) are malignant epithelial mucin-producing tumours encountered in both major and minor salivary glands. The aim of this study was to investigate the histological characteristics of the expression of mucins (MUC1, MUC4, MUC5AC, MUC5B, MUC6) in MECs in search for a possible correlation between qualitative mucin expression and tumour grade. Twelve low-grade, five high-grade MECs and nine normal salivary glands adjacent to tumour tissue were investigated for these mucins by immunohistochemistry. The samples were evaluated with respect to staining pattern and positivity of specific cell types. Normal acinar cells mainly expressed the cytoplasmic mucin MUC5B. MUC1 and MUC4 were expressed in normal ductal cells in approximately half of the samples whereas MUC5AC expression was rare in normal salivary glands. MECs expressed MUC1, MUC4, MUC5AC and MUC5B. The apical membrane of mucous cells lining the cystic cavities showed the strongest staining for MUC1 and MUC4. The expression of MUC4 in mucous cells decreased with increasing histological grade. Expression of salivary mucin MUC5B in mucous cells in low-grade MECs was less intense compared to the expression of MUC5AC in the same cells. In high-grade tumours, a higher expression of MUC5B compared to MUC5AC was noted. In conclusion, MECs express different mucin quantity compared to normal salivary glands. MUC5AC expression in salivary tumour tissue seems to be a metaplastic feature and MUC4 appears to be related to tumour differentiation grade. The relationship between MUC5AC and MUC5B expression could be a useful tool in the diagnosis and estimation of prognosis of MECs.

Key Words: Grading, immunohistochemistry, MUC, mucin, mucoepidermoid carcinoma, salivary gland
SAMMANFATTNING

Mucoepidermoid carcinom (MEC) är en malign mucin-producerande tumör som förekommer i både stora och små spottkött. Syftet med denna studie var att undersöka histologiskt uttryck av muciner (MUC1, MUC4, MUC5AC, MUC5B, MUC6) i MEC för att eventuellt hitta en korrelation mellan kvalitativt mucinuttryck och tumörgrad. Tolv låg- och fem höggradiga MEC och nio normala spottkött intill tumörvävndt undersökt med hjälp av immunohistokemi där proverna utvärderades med avseende på färgningsmönster och positivitet i specifika celltyper. Normala spottkörtelceller uttryckte främst cytoplasmatiskt mucin MUC5B. MUC1 och MUC4 uttrycktes i normala spottkörtelgångsceller i ungefär hälften av proverna medan MUC5AC uttryck var sällsynt i normala spottköttar. MEC:ar uttryckte MUC1, MUC4, MUC5AC och MUC5B. Den apikala delen av membranet i de bägarceller som omger cystiska hålrum visade den starkaste färgningen för MUC1 och MUC4.

Uttryck av MUC4 i bägarceller minskade med ökad histologisk grad. Bägarcellers uttryck av MUC5B:s i låggradig MEC var mindre intensivt än uttrycket av MUC5AC i samma celler. Högre uttryck av MUC5B jämfört med MUC5AC noterades i höggradiga tumörer. Sammanfattningsvis uttrycker MEC olika mängd av muciner än normala spottköttar. MUC5AC:s uttryck i MEC verkar vara en metaplastisk funktion och MUC4 tycks relatera till tumörens differentieringsgrad. Förhållandet mellan MUC5AC och MUC5B uttryck skulle kunna vara ett användbart verktyg vid diagnostisering och prognosutvärdering av MEC.
INTRODUCTION

Epidemiology

Mucoepidermoid carcinomas (MECs) are malignant epithelial mucin-producing tumours encountered in both major and minor salivary glands. They are the most common malignancy in the salivary glands and account for approximately 34, 20 and 30% of the malignant tumours in parotid glands, submandibular glands and minor salivary glands, respectively (1,2). The most frequent site of MECs is the major salivary glands where approximately 60% of these lesions are found, with the parotid gland as the predominant site (48%). In the minor salivary glands, the palate and buccal mucosa are the most common sites (3). On rare occasions, MECs can arise within the facial skeleton, especially the mandible, where about 2% of MECs occur. These lesions appear as a radiolucency within the premolar and molar region and are better known as central or intraosseous MECs (2).

MECs occur over a wide age range with the highest prevalence in the third through fifth decades of life, with a mean age of 46 years. A female predilection (62%) has been noted (1).

Etiology

Little is known about the etiology of MECs, but prior exposure of the head and neck region to ionizing radiation is considered a contributing factor in their development. The origin of MECs is believed to be from reserve cells in inter- and intralobular segments of the salivary duct unit, particularly the central segment (2,3).

Clinical aspects

In the major salivary glands, MECs appear as a solitary, asymptomatic swelling. Patients are often aware of the lesion for approximately a year. In the minor glands, especially the palate, the tumour appears as a fluctuating bluish swelling that can clinically resemble and be mistaken for a mucocele. Symptoms occur in approximately 40% of patients and include pain, dysphagia, ulceration, paresthesia. The tumours can be as large as 12 cm in the major glands and 5 cm in the minor glands (1).

Histological features

The main cellular components of MECs are mucous, intermediate and epidermoid cells (Fig. 2D). Mucous cells are the neoplastic cells in MECs. They are usually large and ovoid with small nuclei compressed near the periphery of the cell, and contain epithelial mucin. They occur in clusters, lining
cystic structures or scattered among other cell types. Intermediate cells range from small basaloid to large epidermoid cells (4). According to a proposed theory of MEC histogenesis, the small basaloid cells are considered as progenitor of mucous, epidermoid and clear cells (Fig 1) (3).

Epidermoid cells are usually scattered all over the tumour mass but may also be arranged in solid nests or islands. There are often basoloid intermediate cells surrounding epidermoid cell islands. Other cell types less predominant in MEC are clear and columnar cells (1, 3, 4). Depending on the arrangement and varying proportions of all cell types, a wide spectrum of differentiation is seen histologically, from cystic to solid tumours. The micro- or macrocysts are usually lined by epidermoid, intermediate or mucous cells. The cystic cavities are often filled with extracellular mucin. Solid tumours are composed mainly of intermediate cells with scattered epidermoid, mucous and clear cells (1).

**Grading system**

In 1945, Stewart et al (5) were the first to classify MECs into benign or malignant tumours according to histological appearance and clinical outcome. In 1953, Foote and Frazell (6) reported that some patients once diagnosed with benign tumour had developed metastases and a new grading system was therefore proposed, dividing MECs into “low-grade” and “high-grade” malignant tumours (Fig. 2 A,C). Because of the large spectrum of histologic appearance existing within MECs, an intermediate-grade was also suggested (Fig. 2B). The difficulty has been to identify those histologic features that are most useful in grading MECs (3). The 3-grade system proposed by Aucalir, Ellis and Goode from the Armed Forces Institute of Pathology (AFIP) (7) has been well accepted among pathologists. The system consists of evaluating and eventually giving points to some histological features: portion of intracystic component, neural invasion, number of mitoses, tumour necrosis and cellular anaplasia. The tumour grade is determined by the sum of the allotted points (8). The problem encountered with this system is the tendency to downgrade MECs (9,10). Prognosis is usually correlated to the grade: low-grade tumours usually having the best prognosis, low recurrence rate and metastatic frequency and high-grade tumours having the worst prognosis with high frequency of recurrence and metastasis (Table 1). However, this cannot be applied to all tumours since there are still some types with a biological behaviour which is hard to predict by grading e.g. submandibular gland MECs. These tumours can metastasize or cause death despite being classified as low-grade (11). Hence there is need for an accepted and easily reproducible method that can predict tumours with potential to recur or metastasize.
**Immunological findings**

Aberrant expression of different forms and amounts of mucins has been observed in a variety of tumours. Transformation and growth of tumour cells is associated with deregulation of mucin core protein expression and a selection process favours expression of those mucins that contribute to cell survival (12). There are two major categories of mucins: membrane-bound mucins (*e.g.* MUC1, MUC4) and secreted mucins (*e.g.* MUC2, MUC5AC, MUC5B, MUC6). A few studies have investigated the expression of mucins in MECs in order to find a relationship between tumour grading and mucin expression that might serve as a better prognostic predictor, but the results have been mixed (13-17).

The aim of this study was to investigate the histological characteristics of mucin expression in mucoepidermoid carcinoma in search for a possible correlation between qualitative mucin expression and tumour grade.

**MATERIALS AND METHODS**

**Case selection**

From the Biobank of the Department of Oral Pathology at the Faculty of Odontology in Malmö, a total of 42 cases diagnosed with mucoepidermoid carcinoma between January 1990 and December 2005, were retrieved. Fourteen of these cases were excluded from the study for reasons such as: missing paraffin-embedded tissues, fine needle aspiration biopsy only etc. The earlier slides of the 28 remaining cases, stained with Haematoxylin-Eosin (HTX) and periodic-acid Schiff (PAS) were reviewed and graded into 12 low-, 11 intermediate- and 5 high-grade MECs. Of the 28 patients included, 8 were male and 20 were female. The mean age of the patients was 50 years, ranging from 12 to 75 years. Only low- and high-grade MECs were chosen to undergo immunohistochemical (IHC) staining. Nine of the selected tumour samples contained normal salivary gland tissue.

**Immunohistochemistry procedure**

The antibodies used in the study were produced at the Dept of Medical Chemistry at Lund University and their features are summarised in Table 2. The dilution for each antibody was established through a series of IHC staining procedures with appropriate positive control tissues. A negative control was included in order to determine specificity. The paraffin-embedded tissues containing from one to five tissue specimens each were cut into 3 μm thick sections and subjected to IHC. The IHC procedure comprised the following main steps: epitope retrieval by heating for 20 minutes at 98°C in triethylene
glycol (TEG) buffer solution, blocking in bovine serum albumin (BSA), application of anti-mucin antibodies at room temperature for 25 minutes, blocking endogenous peroxidase with Dako REAL™ Peroxidase-Blocking Solution and visualization with Dako REAL™ EnVision™ Detection System, (Peroxidase/ DAB +). Slides were counterstained with Mayer's hematoxylin. A positive control was included for all antibodies during each IHC procedure. HTX and PAS samples were also prepared.

For microscopy and visualization, a Nikon Eclipse 80i light microscope equipped with a Nikon digital sight DS-2Mv camera, was used. Normal salivary glands and tumour tissues were evaluated with respect to staining pattern and positivity of specific cell types. Acinar and ductal cells in normal salivary glands and mucous, epidermoid and intermediate cells in MECs were studied. A mark was made on every glass in order to identify exactly the same region of the samples coming from the same tissue. The purpose was to identify and compare the reaction of exactly the same cells against different antibodies in serial sections.

Evaluation of staining was semi-quantitative and qualitative based on the intensity of the reaction in the entirety of the samples and specifically within the marked fields. Due to descriptive nature of the data, no statistical analysis of the staining patterns has been made.

RESULTS

Mucin expression in normal salivary glands

Normal acinar cells in each sample containing normal salivary glands adjacent to tumour tissue (9 of 17 samples) expressed the salivary MUC5B mucin. The other mucins were expressed in acinar cells in only a few or no samples. MUC1 and MUC4 were only/mainly expressed in normal ductal cells in approximately half of the samples. MUC6 was expressed in normal ductal cells of salivary glands in every sample. MUC5AC was the mucin with the lowest expression: only ductal cells in one sample showed positivity. Mucin expression in normal salivary glands was not related to the grade of the adjacent tumour. However, the expression of MUC1 and MUC5AC in tumour tissue was more pronounced than in the adjacent normal salivary gland tissue. The results for mucin expression in normal salivary glands are shown in Fig. 3.
Comparison of staining patterns for MUC1B and MUC214D4 in MECs

The apical membrane of mucous cells in MECs showed positivity for both MUC1B and MUC214D4. Intermediate and epidermoid cells in MECs stained positively with MUC1B antibody (94% and 100% respectively). When using MUC214D4 antibody, there was no positive staining for MUC1 in intermediate cells and 41% of the tumour samples showed positivity in epidermoid cells. Thus there was poor correlation between the two MUC1 antibodies.

The MUC1B antibody used in this study is a relatively new polyclonal antibody that has not been fully evaluated and its reactivity can therefore be questioned. For this reason, only the staining results with the 214D4 antibody will be described in the following paragraphs.

Staining pattern for MUC5B and MUC5AC in MECs

MUC5B and MUC5AC labelled the cytoplasm of mucous cells in tumour tissue. No significant staining was found in intermediate and epidermoid cells. Occasionally, these cells were reactive for MUC5AC and MUC5B but in small numbers and interspersed among unstained cells. Intermediate cells expressed MUC5B in more than 50% of the tumour tissue in two high-grade MECs. These cells were negative for MUC5AC. The extracellular mucin in the cystic cavities often showed positivity for MUC5AC. Expression of MUC5B in mucous cells in low-grade MECs was less intense compared to the expression of MUC5AC in the same cells. Positivity in \(\geq 50\%\) of tumour cells in low-grade MECs was found in 4 samples stained for MUC5B and 10 samples stained for MUC5AC. In high-grade MECs the difference in staining for MUC5B and MUC5AC was also obvious. We noticed a higher expression of MUC5B compared to MUC5AC in tumour cells in high-grade MECs. High-grade MECs showed either negativity or weak positivity for MUC5AC (3 and 2, respectively). However, the same samples showed weak or strong positivity for MUC5B (3 and 2, respectively). Three of five high-grade MECs were positive for MUC5B and negative for MUC5AC. One low-grade MEC showed this relation between MUC5B and MUC5AC expression.

The results of the staining patterns for MUC5B and MUC5AC in tumour cells in MECs are summarised in Table 3. A few representative examples of differences in staining pattern for MUC5AC and MUC5B are shown in Fig. 4.
Staining pattern for MUC1, MUC4 and MUC6 in MECs according to histological grade

Mucous cells expressing MUC1 were found in 75% of low-grade tumours. The majority of stained mucous cells were those lining the cystic cavities. The apical membrane of these cells showed the strongest staining (Fig. 5A). Mucous cells in high-grade MECs showed positivity for MUC1 in two out of five (40%) samples. In one sample, no mucous cells could be identified. The staining was present along the entire cell membrane but was weaker compared to low-grade MECs (Fig. 5B). Staining in epidermoid cells was weaker in both groups of MECs.

Expression of MUC4 in mucous cells was identified in all low-grade MECs. The expression in mucous cells decreased with increasing histological grade (100% respective 40%).

The cytoplasm of epidermoid cells showed strong but diffuse positivity for MUC6. These cells were stained in every sample of tumour tissue. Mucous cells, especially the well-developed ones, expressed no MUC6.

Intermediate cells showed no staining for MUC1 and MUC4. These cells were positive for MUC6 (100%) but the reaction was diffuse. The results of staining for MUC1, MUC4 and MUC6 in mucous and epidermoid cells in low- and high-grade MECs are shown in Fig. 6.

DISCUSSION

Mucins are large, multifunctional glycoproteins, mainly expressed by specialized epithelial cells in the aerodigestive system. They are comprised of two structural classes: transmembrane (membrane-bound) and secreted (gel-forming) mucins. Mucins play important roles in normal tissues: serve as a physical barrier against materials and microorganisms, maintain homeostasis, communicate the status of the extracellular environment to epithelial cells leading to different cellular responses etc. Nevertheless, an important role of mucins is recognized in cancer development and invasion. Like normal epithelial tissue, cancer cells use mucins to control the environment, regulate differentiation and proliferation and enhance invasive and metastatic properties (9). In normal epithelia, mucins are localized to, or secreted from, the apical borders of the cell membrane. Unlike normal epithelial cells, expression of transmembrane mucins, e.g. MUC1 and MUC4 is not restricted to apical borders of tumour cells. The cells loose polarity and mucins are repositioned over the entire cell membrane (18). Various studies on mucin expression, particularly MUC1 and MUC4, in different types of cancers have
found a correlation between mucin expression and prognosis. Aggressive behaviour and poor clinical outcome is associated with expression of MUC1 in papillary thyroid cancers (19) and breast cancer patients with high levels of MUC1 located to non-apical membranes and cytoplasm have a worse prognosis (20). Furthermore, an up-regulated MUC1 expression in human prostate cancer is associated with an increased risk for recurrence (21). MUC4 over-expression as well as loss of confinement to the apical borders of the cells has been identified in carcinomas of ovary, gall bladder, breast, pancreas and lung. Thus, mucins might serve as potential markers for diagnosis of different cancers and early prediction of prognosis in terms of recurrences and survival (22).

In the present study, we investigated mucin expression in mucoepidermoid carcinoma and non-neoplastic salivary gland tissue. Investigators who have studied the expression of mucins in normal salivary glands have reported that besides MUC5B, which is by far the most predominant mucin produced in salivary acinar cells, other mucins as MUC1, MUC4, MUC7 and MUC19 are produced in varying proportions in normal salivary glands (23). In our study we found that: expression of MUC5B in normal salivary glands is restricted to acinar cells and MUC5AC expression is a rare feature in normal salivary glands. MUC1 and MUC4 are only/mainly expressed in epithelial cells lining excretory ducts in approximately half of the samples. Our results are similar to those from previous studies (14, 16, 24). MUC6 was expressed in ductal cells in all samples. Such high expression of MUC6 in non-neoplastic salivary glands has not been reported so far, giving rise to suspicions about the specificity of the antibody against MUC6 used in our study.

Expression of cell associated mucins in MECs has been investigated in previous studies (13-17, 25). It has been reported that high expression of MUC1 is associated with high-grade MECs, high metastasis and recurrences rates and short disease-free period. However, staining for MUC1 has also been proposed to be diagnostic for cases with better prognosis (13,14,16). Alos et al (13) have reported that only 5-10% MUC1-positive tumour cells is sufficient for a poor clinical outcome, although, they have not been able to show a relation between MUC1 expression and any other prognostic indicator (13). In our study, we were also unable to find a relation between MUC1 expression and histological tumour grade. Cases completely lacking MUC1 expression were found in both low- and high-grade MECs.

Expression of MUC4 in MECs is reported to be related to low-grade tumours, reduced risk of death and recurrence rates (13, 25). However, a report from Handra-Luca et al (14) suggests that MUC4
expression is associated with tumour differentiation rather than prognosis and in this study, we could identify a higher expression of MUC4 in low-grade, compared to high-grade MECs. Since we have no clinical follow-up data from the patients, we cannot draw any conclusions regarding a possible relationship between MUC4 expression and prognosis of disease.

MUC5AC is a type of gel-forming gastric mucin, expressed in mucous cells in normal gastric mucosa. However, in many precancerous and malignant lesions, MUC5AC is highly expressed. This means a significant up-regulation of MUC5AC gene transcription (26) and therefore, expression of the secreted mucin MUC5AC in salivary tumour tissue seems to be a metaplastic feature in relation to the lack of expression in normal salivary glands. In this study, we found that expression of MUC5AC was less intense and the proportion of positive cells was smaller in high-grade MECs compared to low-grade MECs. In contrast, expression of MUC5B was identified in almost all the samples (16/17) but the intensity of staining and the proportion of stained tumour cells in low-grade MECs was smaller compared to MUC5AC expression in the same cells. Thus, MUC5AC expression overshadows MUC5B expression in low-grade MECs. The opposite was found for high-grade MECs where MUC5B expression was higher than MUC5AC expression. We speculate that higher expression of MUC5B compared to MUC5AC in MECs can be related to low differentiation. In low-grade MECs, a high differentiation of tumour cells is noted. This is combined with a down-regulation of the MUC5B gene in favour of MUC5AC production. In high-grade MECs, because of a poorer differentiation of tumour cells, MUC5AC expression is less obvious, sometimes totally missing, and MUC5B production takes over. We believe that the relationship between MUC5B and MUC5AC expression could be a useful tool in diagnosis and prognosis of MECs. However, one has to be cautious in drawing parallels between our results and clinical prognosis, because of the lack of data about the clinical outcome of the patients.

Secreted mucin MUC6 was expressed in epidermoid and intermediate cells but no positivity was found in mucous cells. Once again, such strong positivity for MUC6 in tumour cells has not been reported in previous studies. We believe that this may depend on the low specificity of the anti- MUC6 antibody. In our positive controls, we could notice a strong positivity in mucous cells of the gastric mucosa but also a less intense positivity in other types of cells that normally should not be stained for MUC6. This might explain the strong but not specific positivity in epidermoid and intermediate cells in MECs.
In conclusion, studying mucin expression in mucoepidermid carcinoma of salivary glands may provide an important tool for early prediction of prognosis in terms of recurrences and survival. MECs show an alteration of mucin expression compared to normal salivary glands. MUC1, MUC4, MUC5AC are over-expressed in tumour cells of MECs. MUC4 is related to tumour differentiation grade. MUC5AC seems to be a metaplastic feature. The relationship between MUC5AC and MUC5B expression could be a useful tool in diagnosis and prognosis of MECs.

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REFERENCES


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TABLES

**TABLE 1.** Clinical outcome of MECs depending on histological grade (11).

<table>
<thead>
<tr>
<th></th>
<th>Low-grade</th>
<th>Intermediate-grade</th>
<th>High-grade</th>
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<tr>
<td>Local recurrence</td>
<td>6%</td>
<td>20%</td>
<td>80%</td>
</tr>
<tr>
<td>Metastasis</td>
<td>exceptional</td>
<td>rare</td>
<td>frequent</td>
</tr>
<tr>
<td>5 year-survival</td>
<td>95%</td>
<td>80-90%</td>
<td>25-30%</td>
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**TABLE 2.** Antibodies used in the study.

<table>
<thead>
<tr>
<th>Antigen/Antibody</th>
<th>Origin</th>
<th>Sequence</th>
<th>Positive control tissue</th>
<th>Labeling</th>
<th>Dilution</th>
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</thead>
<tbody>
<tr>
<td>MUC1/MUC1B</td>
<td>Polyclonal/rabbit/ C-terminal</td>
<td>CRRKNYGQLDIFPARD</td>
<td>Breast cancer</td>
<td>Membrane-bound</td>
<td>1/2000</td>
</tr>
<tr>
<td>MUC1/214D4</td>
<td>Monoclonal/mouse/ N-terminal</td>
<td>---------------------------</td>
<td>Breast cancer</td>
<td>Membrane-bound</td>
<td>1/500</td>
</tr>
<tr>
<td>MUC4/MUC4-8</td>
<td>Polyclonal/rabbit/ C-terminal</td>
<td>WNNNPEDDFRMPC</td>
<td>Adenocarcinoma from pancreas</td>
<td>Membrane-bound</td>
<td>1/2000</td>
</tr>
<tr>
<td>MUC5AC LUM5-1</td>
<td>Polyclonal/rabbit/ mucin domain</td>
<td>RQQDQQGPKFMC</td>
<td>Normal gastric mucosa</td>
<td>Cytoplasmic</td>
<td>1/2000</td>
</tr>
<tr>
<td>MUC5B/LUM5B-2</td>
<td>Polyclonal/rabbit/ mucin domain</td>
<td>RNRWQVGKFMC</td>
<td>Normal salivary gland</td>
<td>Cytoplasmic</td>
<td>1/8000</td>
</tr>
<tr>
<td>MUC6/LUM6-3</td>
<td>Polyclonal/rabbit/ mucin domain</td>
<td>RPLHSYEQQLELPC</td>
<td>Normal gastric mucosa</td>
<td>Cytoplasmic</td>
<td>1/4000</td>
</tr>
</tbody>
</table>

**TABLE 3.** MUC5B and MUC5AC immunoreactivity in tumour cells in low- and high-grade MECs.

<table>
<thead>
<tr>
<th></th>
<th>MUC5B</th>
<th>MUC5AC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low-grade</td>
<td>High-grade</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>&lt;50% of tumour cells</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>≥50% of tumour cells</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>N = 12</td>
<td>N = 5</td>
</tr>
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</table>
FIGURE 1. Proposed histogenesis of MEC (modified from 3).

FIGURE 2. A. Appearance of low-grade MEC. Note the mucous cells (arrow) lining the cystic cavities. B. Intermediate-grade MEC. C. A solid high-grade MEC mainly containing intermediate cells. D. A mixture of mucous cells (1), intermediate cells (2) and epidermoid cells (3) in low-grade MEC. (HTX, original magnification A,B,C x 20; D x 40)
FIGURE 3. Expression of mucins in acinar and ductal cells of normal salivary glands adjacent to tumour tissue (n = 9 patients).

FIGURE 4. Representative examples of differences in staining pattern for MUC5B and MUC5AC.
A. A section from a low-grade MEC stained for MUC5B. Note a weak positively stained mucous cell (arrow).
B. A serial section from the same low-grade MEC (A) stained for MUC5AC. The mucous cells show strong positivity for MUC5AC (arrow).
C. A high-grade MEC positively stained for MUC5B.
D. The same high-grade MEC (C) negatively stained for MUC5AC. (original magnification x 40).
FIGURE 5. Representative examples of staining pattern for MUC4 in low-grade MEC (A) and high-grade MEC (B). MUC4 stains the apical membrane of mucous cells in low-grade MEC (arrow in A). The staining in high-grade MEC is also specific but weak (arrow in B). (original magnification x 40).

FIGURE 6. Staining pattern for MUC1, MUC4 and MUC6 in mucous and epidermoid cells of MECs according to microscopic grade (n = 12 patients).